Unsaturated Fatty Acids promote carotenoid bioavailability *in vitro*

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ABSTRACT

Dietary lipid is a known enhancer of carotenoid bioavailability, although information on the amounts and types of lipid on optimal absorption of these compounds is limited. We have investigated the effects of several common commercial lipids on carotenoid bioavailability using in vitro digestion and Caco-2 human intestinal cells. Meals consisted of a mixed vegetable salad (3g) with test lipid (180mg). Micellarization and cellular uptake of β-carotene (βC) and lycopene during small intestinal digestion was increased by lipids rich in unsaturated FA: soybean oil > olive oil > canola oil > butter. In contrast, type of lipid minimally affected micellarization or cellular uptake of xanthophylls. Caco-2 cells grown on transwell membrane were chronically exposed to micellar mixtures of FA (1.0 mM) mimicking the types and ratio of saturated to unsaturated (mono- + poly-unsaturated) FA present in butter (70:30), olive oil (7: 93) and soybean oil (11: 89). Then cells were exposed to micelles containing βC, LUT and a mixture of FA. Uptake and transepithelial transport of βC and LUT were greater in cells pre-treated with mixtures enriched in unsaturated compared to saturated FA. These results suggest that oils rich in unsaturated FA promote carotenoid bioavailability. (USDA NRI and OARDC)

INTRODUCTION

- The bioavailability of carotenoids, which are the major dietary source of pro-vitamin A, is generally low.
- Factors affecting bioavailability are, but not limited to, 1) Physico-chemical properties of carotenoids, 2) Food matrix (processing and formulation), 3) Nutritional and physiological status and 4) genotype
- Evidences showed dietary lipid incorporation into meal can help the bioaccessibility and bioavailability [1, 2]
- However, data on the effect of dietary lipid type and fatty acid profile (i.e. saturated vs unsaturated) to the bioavailability of carotenoids is limited.

HYPOTHESIS

Different profile of dietary fatty acid can affect to the bioaccessibility and bioavailability of carotenoids

Figure 1: Chemical Structure of Carotenoids

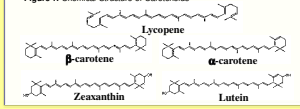
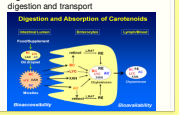


Figure 2: Schematic of carotenoid digestion and transport



OBJECTIVES

- To develop strategies for enhanced utilization of carotenoids from commonly consumed foods
- Examine impact of dietary fat profiles (TG fatty acyl chain length, degree of saturation) on
  1) micellarization,
  2) intestinal cell uptake, and
  3) chylomicron mediated transport of carotenoids.

MATERIALS & METHODS

- **Food Model:** Salad was selected as a standard dietary model for the study. All ingredients were obtained from standard grocery store and then weighed according to normal salad proportion by USDA [3] (Table 1). The salad was homogenized and pureed by commercial blender for 2 minutes.

Table 1: Salad Recipe

Vegetable	Weight (g)
Tomatoes	95
Carrot	62
Spinach	50
Romaine Lettuce	26
Orange Pepper	23

Table 2: Calculated content of carotenoids in Salad Recipe

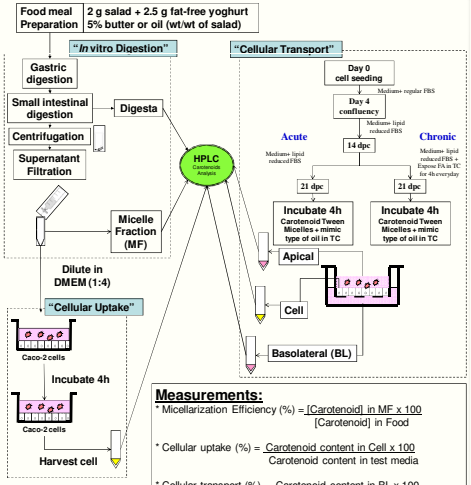
Carotenoid	Content (μg/g)
Lutein	15.2
Zeaxanthin	5.3
α- Carotene	15.7
β-Carotene	22.1
cis-β-Carotene	1.0
Lycopene	22.0
cis-Lycopene	1.4

Table 3: Typical fatty acids profile of edible fat & oils

		Butter	Olive oil	Canola oil	Soybean oil
Caprylic acid	(C8:0)	5.0			
Capric acid	(C10:0)	2.6			
Lauric acid	(C12:0)	4.5			
Myristic acid	(C14:0)	**14.2**			
Palmitic acid	(C16:0)	**30.2**	11.8	4.0	**11.0**
Stearic acid	(C18:0)	10.5		1.8	3.8
Palmitoleic acid	(C16:1)	5.7	1.6		0.2
Oleic acid	(C18:1)	**18.7**	**72.0**	**56.1**	**22.8**
Linoleic acid	(C18:2)		9.2	**30.3**	**51.0**
α-Linolenic acid	(C18:3)		0.8	9.3	6.8

Figure 3: Typical consumption of carotenoid in western diet (Photo Source: http://www.pacificspecialty.com/foodfreecipe/recipes_mango2004.html)

- **Experimental Design:**



Measurements:

* Micellarization Efficiency (%) = [Carotenoid] in MF x 100 / [Carotenoid] in Food

* Cellular uptake (%) = Carotenoid content in Cell x 100 / Carotenoid content in test media

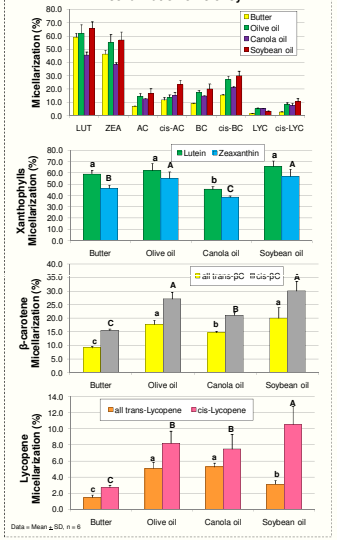
* Cellular transport (%) = Carotenoid content in BL x 100 / Carotenoid content in Cell and BL

- **Chemicals and supplies:** all chemical and supplies were purchased from Sigma-Aldrich and Fisher Scientific.
- **Extraction and analysis of carotenoids:** Food sample and digesta were saponified by adding equal amount of 30% KOH solution and incubate (37oC 30 minutes) to remove chlorophylls. The harvested cells were sonicated in PBS solution for 30 seconds prior to extraction. The extraction was conducted by using Tetrahydrofuran (THF): Hexane (1:1) repeatedly until clear solution obtained. Centifuged upper layer was collected and evaporated under nitrogen gas stream until dried and resolubilized with Methanol : Methy-tert butyl ether (MTBE) (1:1) for HPLC injection.
- **HPLC analysis:** A system of coupled Waters 2695 separation module with Water 2996 Photodiode Array (PDA) at 450 nm was used for separation and detection of carotenoids. Column: YMCTM Carotenoid S-5 C30 reversed-phase (150 mm x 4.6 mm, 5μm). Mobile Phase: (A) = Methanol : Ammonium acetate (98:2), (B) = MTBE.
- **In vitro digestion:** detail of chemical preparation and conditions used in simulated digestion adopted from previous report. [4]
- **Cellular uptake and transport by Caco-2 cells:** Cell culture media preparation and cell maintenance procedure adopted from previous report.[5]

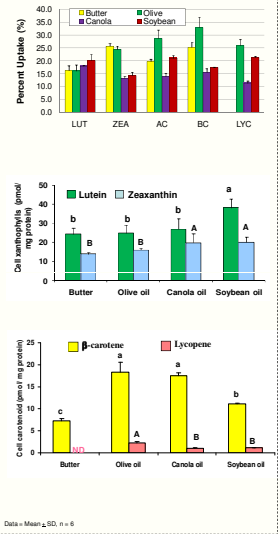
RESULTS

Photo Source: http://www.mcgacanola.org/food_photography.html

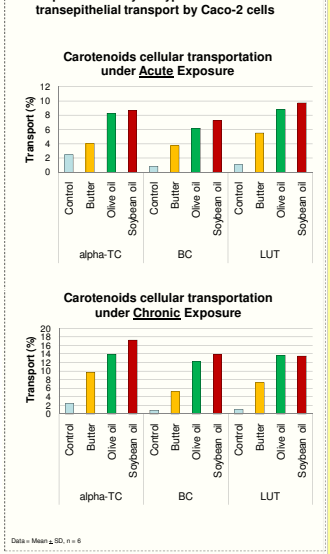
Bioaccessibility: Micellarization Efficiency



Cellular Uptake



Bioavailability: Cellular Transport



CONCLUSIONS

- Micellarization and cellular uptake of β-carotene (βC) and lycopene during small intestinal digestion was increased by lipids rich in unsaturated FA: soybean oil > olive oil > canola oil > butter. In contrast, type of lipid minimally affected micellarization or cellular uptake of xanthophylls.
- Uptake and transepithelial transport of βC and LUT were greater in cells pre-treated with mixtures enriched in unsaturated compared to saturated FA.

SUMMARY

Oils rich in unsaturated FA may promote carotenoid bioavailability.

ACKNOWLEDGEMENTS

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